Adenosine gates synaptic plasticity at hippocampal mossy fiber synapses

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The release properties of synapses in the central nervous system vary greatly, not only across anatomically distinct types of synapses but also among the same class of synapse. This variation manifests itself in large part by differences in the probability of transmitter release, which affects such activity-dependent presynaptic forms of plasticity as paired-pulse facilitation and frequency facilitation. This heterogeneity in presynaptic function reflects differences in the intrinsic properties of the synaptic terminal and the activation of presynaptic neurotransmitter receptors. Here we show that the unique presynaptic properties of the hippocampal mossy fiber synapse are largely imparted onto the synapse by the continuous local action of extracellular adenosine at presynaptic A1 adenosine receptors, which maintains a low basal probability of transmitter release.

The basic physiological properties of synapses in the brain exhibit a remarkable diversity, most of which is due to the type of transmitter released and the compliment of receptor subtypes clustered pre- and postsynaptically. On the other hand, the presynaptic release of transmitter appears to rely on a rather stereotyped set of proteins. Even so, it is well established that synapses can differ considerably in terms of their response to repeated activation. For instance, most excitatory synapses exhibit paired-pulse facilitation (PPF), in which the second response to two closely timed stimuli (e.g., 40 ms) is modestly enhanced (1), whereas inhibitory synapses typically exhibit paired-pulse depression (2). The hippocampal mossy fiber synapse is unlike most other synapses in the central nervous system because of its dramatic PPF (3, 4). Another defining characteristic of the hippocampal mossy fiber synapse is its remarkable frequency facilitation, where changing the frequency of stimulation from a low rate (e.g., 0.05 Hz) to a modest rate (e.g., 1 Hz) enhances transmission manyfold. This feature is in striking contrast to neighboring associational/commissural (A/C) synapses, where little frequency facilitation occurs (4).

This heterogeneity in activity-dependent transmitter release is thought to depend primarily on the basal release probability. Thus, the large PPF and frequency facilitation at the mossy fiber synapse require that during resting conditions the probability of release is extremely low. The probability of release can vary greatly in the same class of synapses (1, 5, 6), even among synapses arising from the same neuron (6–8). Previous research has found that extracellular adenosine acting on presynaptic inhibitory adenosine A1 receptors can affect transmitter release (5, 9). Here we report that the low release probability at the mossy fiber synapse results from tonic activation of presynaptic A1 receptors by ambient adenosine. Thus, removal of the adenosine tone, either by enzymatically degrading extracellular adenosine or by removing A1 receptor function, dramatically enhances mossy fiber synaptic transmission and largely occludes frequency facilitation. In addition, mossy fiber long-term potentiation (LTP), an *N*-methyl-D-aspartate receptor-independent presynaptic form of plasticity, is also impaired. Thus, many of the defining features of the mossy fiber synapse are dependent on the continuous activation of A1 receptors.

Methods

As described, transverse rat and mouse hippocampal slices (400-μm-thick) were prepared in artificial cerebrospinal fluid (ACSF) (10) containing a high concentration of sucrose, and then incubated in normal ACSF (containing 119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 11 mM glucose and equilibrated with 95% O_2/5% CO_2 at room temperature) for at least 1 h before recording. In some cases, slices were cut in normal ACSF, rather than in high-sucrose ACSF. Regardless of the method of slice preparation, results were the same. Slices were transferred to a recording chamber mounted on an Olympus BX50 microscope equipped for IR-differential interference microscopy. To minimize polysynaptic activity, slices were superfused (3–4 ml/min) continuously in the recording chamber with ACSF containing 4 mM Ca^{2+} and 4 mM Mg^{2+}.

Field excitatory postsynaptic potentials (fEPSPs) were recorded extracellularly at room temperature (unless otherwise noted) by using low-resistance patch pipettes filled with 1 M NaCl. For mossy fiber fEPSPs, a bipolar platinum-stimulating electrode was placed in the hilus directly adjacent to the granule cell layer and the recording electrode was placed in stratum lucidum. To confirm that the fEPSPs were mossy fiber in origin and to isolate the presynaptic fiber volley, a group 2 metabotropic glutamate receptor (mGluR) agonist, (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (1 μM) (11) or (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine (LCCG-1) (10 μM), was applied at the end of each experiment. For A/C and Schaffer collateral fEPSPs, the stimulating and recording electrodes were placed in stratum radiatum of CA3 and CA1, respectively, and 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxaline (25 μM) was applied at the end of each experiment to assess the fiber volley component. Baseline stimulation frequency was 0.05 Hz. Synaptic responses were filtered at 2 kHz and digitized at 5 kHz. Data were collected and analyzed by using IGOR PRO software. All data are expressed as mean ± SEM. The following drugs, prepared daily from concentrated (≥1,000×) stock solutions, were used in this study: *N*-ethylmaleimide (NEM, Sigma), LY341495 (Tocris Cookson, Ellisville, MO), naloxone (Tocris Cookson), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (Tocris Cookson), adenosine deaminase (Sigma), (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCGIV) (Tocris Cookson), LCCG-1 (Tocris Cookson), baclofen (Sigma), 2-chloro-N^6-cyclopentyladenosine (CCPA, Sigma), 3-[(±)-2-carboxypiperazin-4-yl]propyl-1-phosphonic acid (Sigma), and 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxaline (Tocris Cookson).

Abbreviations: PPF, paired-pulse facilitation; A/C, associational/commissural; LTP, long-term potentiation; ACSF, artificial cerebrospinal fluid; fEPSP, field excitatory postsynaptic potential; LCCG-1, (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; NEM, *N*-ethylmaleimide; CCPA, 2-chloro-N^6-cyclopentyladenosine; $GABA_B$, γ-aminobutyric acid type B; mGluR, metabotropic glutamate receptor.

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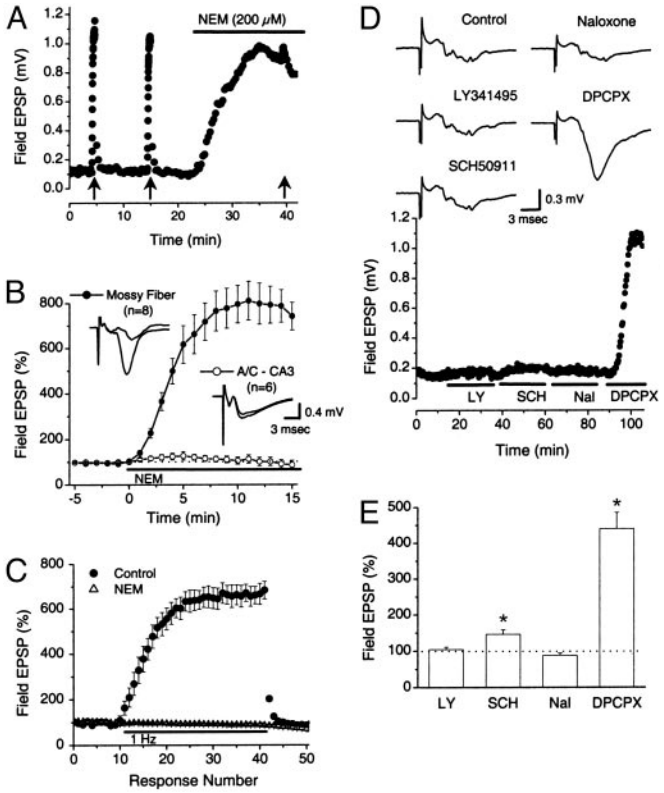

Fig. 1. NEM and DPCPX greatly enhance mossy fiber transmission. (*A*) Time course of a typical experiment illustrating the effect of NEM (200 μM), a sulfhydryl-alkylating agent that blocks G_i/G_o-coupled receptors, on mossy fiber transmission and 1-Hz frequency facilitation. At the arrows, the stimulation frequency was increased from 0.05 to 1 Hz. (*B*) Summary of the effects of NEM on mossy fiber and A/C fEPSPs. Mossy fiber fEPSPs are significantly ($P <$ 0.0001) increased by NEM, whereas A/C transmission is first slightly enhanced then depressed. Sample traces illustrating the effects of NEM (10 min) on mossy fiber and A/C transmission are also shown. (*C*) The effect of NEM on mossy fiber frequency facilitation is illustrated on an expanded timescale. Under control conditions, increasing the stimulation frequency from 0.05 to 1 Hz (indicated by the arrows in *A*) facilitates mossy fiber fEPSPs in a reversible fashion. In the presence of NEM, 1-Hz frequency facilitation is blocked. (*D*) A series of G protein-coupled receptor antagonists were screened in sequence for their ability to mimic the effect of NEM on baseline mossy fiber transmission. Only DPCPX (200 nM), a selective adenosine A1 receptor antagonist, was able to recapitulate the NEM effect. A broad-spectrum metabotropic glutamate receptor antagonist, LY341495 (20 μM), and an opioid receptor antagonist, naloxone (10 μM), had no effect, whereas the $GABA_B$ receptor antagonist, SCH50911 (10 μM), slightly enhanced transmission. Sample mossy fiber fEPSP traces shown above the summary time course were recorded before and during antagonist application. The results of four such experiments are summarized in *E*. LY, LY341495; Nal, naloxone; SCH, SCH50911. *, $P < 0.05$ vs. baseline, Student's *t* test.

Results

In studying the possible role of presynaptic receptors in the control of mossy fiber synaptic transmission, we applied the sulfhydryl-alkylating agent NEM, which has been shown to block $G_{i/o}$ coupled receptors (12, 13), a common class of presynaptic inhibitory receptor. In this experiment we first established that the responses were mediated by mossy fibers, in that they exhibited large frequency facilitation. Application of NEM caused a dramatic increase in the mossy fiber response (Fig. 1 *A* and *B*, filled circles; $P < 0.0001$), whereas the response to activation of the neighboring A/C synapses was little affected (Fig. 1*B*, open circles). Furthermore, in the presence of NEM, mossy fiber frequency facilitation was entirely blocked (Fig. 1 *A* and *C*). Although NEM has many actions in addition to blocking $G_{i/o}$ these results raise the possibility that mossy fiber synaptic transmission is tonically inhibited by a $G_{i/o}$-mediated process and that enhancement of transmitter release occludes frequency facilitation. It is well established that the inhibitory action of several presynaptic metabotropic receptors is mediated by $G_{i/o}$ at mossy fiber synapses. These include mGluRs, κ-opioid receptors, γ-aminobutyric acid type B ($GABA_B$) receptors, and adenosine A1 receptors. Previous studies have shown that blockade of mGluRs has no effect on baseline transmission, and blockade of $GABA_B$ causes only a modest increase (14, 15). We have confirmed these results in the present study, in which the broad-spectrum mGluR antagonist LY341495 (20 μM) had no effect, and the selective $GABA_B$ receptor antagonist SCH50911 (10 μM) enhanced baseline responses to 146 ± 12% ($n = 4$; $P =$ 0.0086) (Fig. 1 *D* and *E*). In addition, the broad-spectrum opioid antagonist naloxone (10 μM) did not enhance baseline responses (Fig. 1 *D* and *E*). In striking contrast, in the same experiments, application of the adenosine A1 receptor antagonist DPCPX (200 nM) caused a dramatic increase in the mossy fiber responses to 440 ± 45% ($n = 4$; $P = 0.0003$) (Fig. 1 *D* and *E*), suggesting that the effects of NEM were caused by the removal of A1 inhibition of mossy fiber transmission.

The enhancement in mossy fiber fEPSPs by DPCPX application is significantly greater than the enhancement observed at neighboring A/C synapses and at Schaffer collateral synapses in the CA1 region (Fig. 2*A*; $P < 0.0001$), suggesting that under normal conditions, extracellular adenosine potently inhibits mossy fiber synaptic transmission by activating A1 receptors. Enhancement of mossy fiber transmission by DPCPX was equally robust at physiological temperature (507 ± 40% at 24°C ($n = 27$) vs. 582 ± 100% at 35–37°C ($n = 6$; $P = 0.444$). Application of adenosine deaminase, which degrades extracellular adenosine, has been reported to cause ≈50% enhancement of excitatory synaptic transmission in the CA1 region of the hippocampus (16). We find that adenosine deaminase causes a large enhancement in mossy fiber synaptic transmission (Fig. 2*B*; $P < 0.0001$) but has little effect on the neighboring A/C excitatory synapses. Similarly, adenosine deaminase had no effect on mossy fiber synaptic transmission in the adenosine A1 receptor knockout mouse. This finding strongly suggests that extracellular adenosine tonically inhibits mossy fiber transmission by activating A1 receptors. This conclusion is further supported by comparing synaptic strength in wild-type and A1 knockout mice. In an initial nonblind study we found that for any given presynaptic strength (fiber volley amplitude), the ratio of fEPSPs (output) in A1 knockouts versus wild-type mice was ≈5. To confirm this observation, we measured the input–output relationship in the two types of mice in a blind fashion (Fig. 2*C*). A comparison of the curves indicates that synaptic strength is greatly enhanced in the absence of A1 receptors ($P \leq 0.02$). This augmentation was not due to differences in the degree of nonmossy fiber contamination because the depression caused by (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine, which selectively depresses mossy fiber responses (11), was the same in both groups (wild type = 88 ± 4.8%, $n = 5$; knockout = 95 ± 1.3%, $n = 5$).

As discussed above, mossy fiber synapses exhibit pronounced frequency facilitation and PPF. Fig. 3*A* shows frequency facilitation at mossy fiber synapses and the effects of applying DPCPX. Baseline synaptic responses were collected at a frequency of 0.05 Hz. At the arrows, the frequency was increased to 1 Hz for 30 s. The responses rapidly increase and recover from the change in stimulus frequency. DPCPX was then applied, which markedly enhanced transmission and greatly reduced frequency facilitation. At the end of the experiment, the group 2 selective mGluR agonist LCCG-1 was applied, and it blocked the response. The sensitivity to LCCG-1 confirms that the

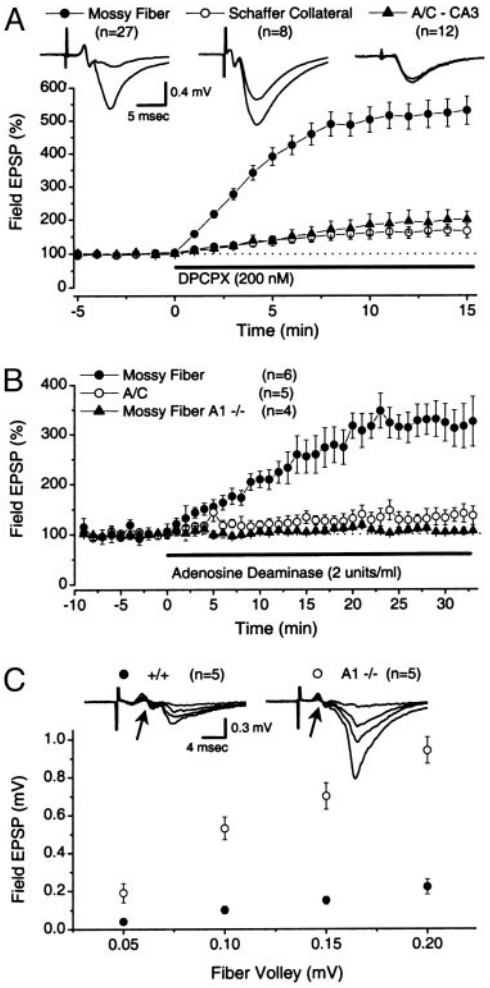

Fig. 2. Removing tonic adenosine A1 receptor activation enhances mossy fiber transmission. (*A*) Bath application of DPCPX, an A1 receptor antagonist, has a significantly greater effect on mossy fiber synapses than on neighboring A/C synapses ($P < 0.0001$) and more distant Schaffer collateral synapses ($P < 0.0001$). Representative fEPSPs recorded before and during DPCPX application from each of the three types of synapses are shown above the summary time course. (*B*) Adenosine deaminase, which enzymatically degrades adenosine, also boosts the baseline level of mossy fiber transmission in wild-type mice ($P < 0.0001$) but has no effect in littermates with the A1 receptor knocked out ($-/-$). Similar to DPCPX, adenosine deaminase has little effect on transmission mediated by A/C fibers. (*C*) Basal mossy fiber transmission is enhanced in A1 receptor knockout ($-/-$) mice compared with wild-type ($+/+$) littermates. The amplitude of mossy fiber fEPSPs evoked by a range of stimulus intensities is plotted against the amplitude (measured peak to peak) of the corresponding fiber volley. The fiber volley is indicated by the arrow. As illustrated in the sample traces and the summary below, for each input (fiber volley), the output (fEPSP) is significantly greater in slices from A1 $-/-$ mice ($P \leq 0.02$).

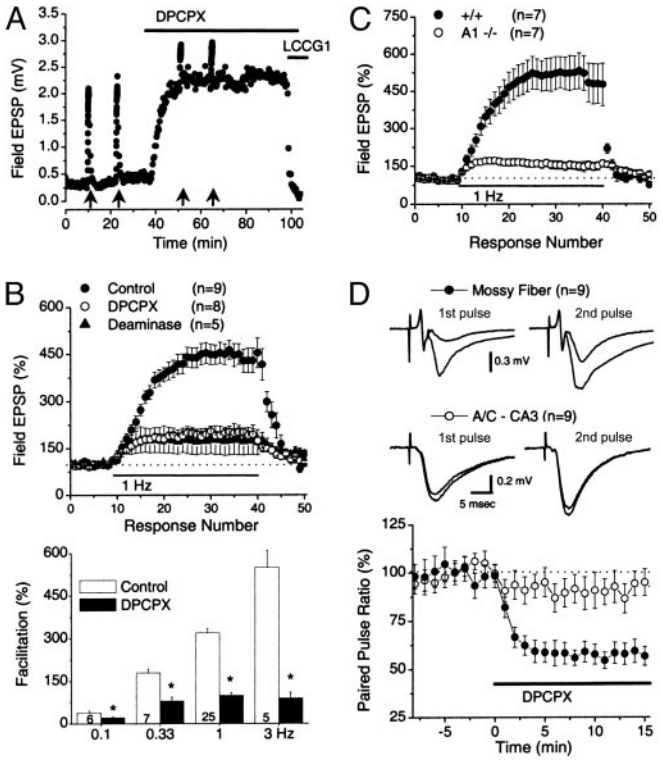

Fig. 3. A1 receptor activation is essential for short-term mossy fiber plasticity. (*A*) Time course of a typical experiment illustrating the effect of DPCPX (200 nM) on mossy fiber transmission and 1-Hz frequency facilitation. At the arrows, stimulus frequency was increased from 0.05 to 1 Hz. Under baseline conditions, increasing the stimulation frequency to 1 Hz increases the response amplitude by 425%. In the presence of DPCPX (200 nM), facilitation is decreased to only 35% above baseline. In this and all mossy fiber experiments, LCCG-1 (10 μM) or (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (1 μM) was applied to confirm that fEPSPs are of mossy fiber origin. (*B*) Upper graph shows 1-Hz frequency facilitation (indicated by arrows in *A*) illustrated on an expanded timescale. After a 15- to 30-min bath application of DPCPX or adenosine deaminase (2 units/ml) to rat hippocampal slices, 1-Hz frequency facilitation is significantly reduced ($P < 0.0001$). Lower graph shows a summary of mossy fiber frequency facilitation evoked at a variety of stimulation frequencies before and during DPCPX application. Numbers of experiments are indicated for each frequency. *, $P < 0.05$, paired *t* test. (*C*) A 1-Hz frequency facilitation was also significantly ($P < 0.0001$) attenuated in A1 receptor knockout ($-/-$) mice compared with wild-type ($+/+$) littermates. (*D*) In the presence of DPCPX, mossy fiber PPF (40-ms interstimulus interval) was significantly reduced ($P < 0.0001$). In contrast, PPF at A/C synapses was not significantly affected. The sample traces above illustrate the robust PPF typically observed at mossy fiber synapses and the ≈50% reduction in DPCPX. PPF at A/C synapses, which is characteristically much smaller, is also illustrated.

responses are generated by mossy fiber synapses. A summary of several experiments in which either DPCPX or adenosine deaminase was applied is graphed in Fig. 3*B Upper*. The responses in the presence of DPCPX and adenosine deaminase have been normalized to 100%. Both manipulations significantly reduced the frequency facilitation ($P < 0.0001$). The depressant effect of DPCPX on frequency facilitation was present at all frequencies (Fig. 3*B Lower*). We have also compared frequency facilitation in wild-type and A1 knockout mice (Fig. 3*C*). Again, the frequency facilitation is significantly reduced in the absence of A1 receptors ($P < 0.0001$). The enhancement in synaptic transmission at mossy fiber synapses by DPCPX was also accompanied by a large decrease in PPF (Fig. 3*D*; $P < 0.0001$). This finding and the effects observed on frequency facilitation imply that removing extracellular adenosine, deleting A1 receptors, or pharmacological blockade of the A1 receptor causes a dramatic increase in the probability of transmitter release at the mossy fiber synapse. Thus, under normal conditions, ambient adenosine strongly inhibits the probability of transmitter release at mossy fiber synapses by activating presynaptic A1 receptors.

In addition to the pronounced short-term presynaptic plasticity observed at mossy fiber synapses, these synapses also exhibit an unusual presynaptic form of LTP (17, 18). Mossy fiber LTP is independent of *N*-methyl-D-aspartate receptor activation and is expressed as a long-lasting enhancement in the probability of transmitter release. Given that presynaptic A1 receptor activation has such a profound effect on the probability of transmitter release at mossy fiber synapses, one might expect mossy fiber LTP to be strongly influenced by extracellular adenosine. In-

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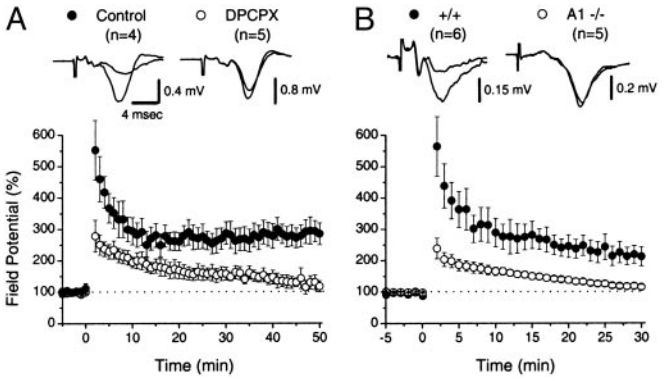

Fig. 4. Removal of adenosine A1 receptor action impairs mossy fiber LTP. (*A*) Tetanic stimulation (three trains of 25 Hz for 5 sec, every 10 sec) at time = 0 produced robust posttetanic potentiation and LTP under control conditions, both of which were significantly ($P = 0.0057$) reduced by bath application of DPCPX (20–30 min). Sample traces represent the average of the 5 min before the tetanus and the 40–45 min after the tetanus. (*B*) Similarly, posttetanic potentiation and LTP are significantly ($P = 0.0046$) reduced in A1 −/− mice compared with wild-type littermates. Summary traces from before and 25–30 min after the tetanus are shown above. An *N*-methyl-D-aspartate receptor antagonist, 3-[(±)-2-carboxypiperazin-4-yl]propyl-1-phosphonic acid (10 μM), was present throughout all LTP experiments.

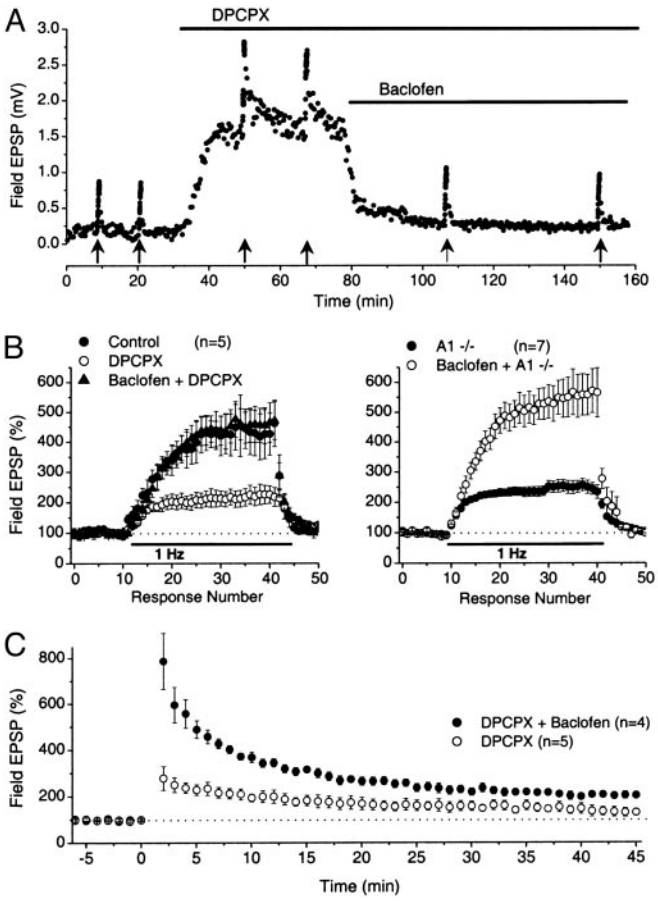

Fig. 5. Activation of presynaptic $GABA_B$ receptors can substitute for A1 receptors. (*A*) A typical experiment showing that the effect of DPCPX on frequency facilitation (1-Hz stimulation at the arrows) can be completely reversed by the addition of the $GABA_B$ receptor agonist baclofen (0.2 μM). (*B*) A summary of the effects of baclofen on frequency facilitation in the presence of DPCPX (*Left*) or in slices from A1 −/− mice (*Right*). For ease of comparison, responses were renormalized to 100% after baclofen application. In the presence of baclofen, frequency facilitation was restored to levels typically observed in wild-type mice. (*C*) Application of baclofen, after DPCPX application, to reduce mossy fiber transmission back to control levels also restores mossy fiber posttetanic potentiation and LTP. DPCPX data are replotted from Fig. 4.

deed, in the presence of DPCPX, mossy fiber LTP is markedly depressed (Fig. 4*A*; $P = 0.0057$, 40–50 min after tetanus). A similar depression is also seen in slices from A1 knockout mice (Fig. 4*B*; $P = 0.0046$, 25–30 min after tetanus).

Are the effects of A1 receptor activation on short- and long-term plasticity at the mossy fiber synapse unique to A1 receptors? To answer this question, we tried to rescue these properties after removal of A1 receptor action on the mossy fiber synapse by applying an agonist of $GABA_B$ receptors, another presynaptic $G_{i/o}$ receptor (Fig. 5*A*). First, we compared frequency facilitation before and after blocking A1 receptors with DPCPX. In Fig. 5*A*, the control frequency facilitation was ≈4-fold and after DPCPX it was <2-fold. We then depressed synaptic transmission back to control values with the $GABA_B$ receptor agonist baclofen. In the presence of DPCPX, baclofen restored frequency facilitation. Fig. 5*B* (left-hand graph) summarizes the experiments showing the interaction of baclofen (0.2–1 μM) with DPCPX. The reduction in frequency facilitation caused by DPCPX is completely reversed by baclofen. Similarly, baclofen was able to completely restore frequency facilitation in the A1 receptor knockout mouse (Fig. 5*B*, right-hand graph). Finally, robust mossy fiber LTP was evoked when baclofen was coapplied with DPCPX (Fig. 5*C*).

Can the difference in the magnitude of frequency facilitation between mossy fiber and nonmossy fiber synapses be explained entirely by the difference in A1 inhibitory tone? This does not appear to be the case, because frequency facilitation of CA1 synapses is only modestly enhanced in the presence of exogenously applied adenosine. Addition of adenosine (10–30 μM), which depressed transmission by ≈80%, only increased 1-Hz frequency facilitation from 1.12 ± 0.04 to 1.55 ± 0.05 ($n = 3$). Thus, it appears that, although the presence of adenosine is necessary for the robust frequency facilitation at mossy fiber synapses, it is not sufficient. The contribution of presynaptic kainate receptors to frequency facilitation at mossy fiber synapses (19–21) can account for some, but not all, of this difference.

Why are the effects of ambient adenosine so powerful and selective for mossy fiber synapses? We considered several possibilities. It is unlikely that different types of adenosine receptors are involved because the effects are absent in the A1 receptor knockout mouse. We also considered the possibility that properties of the A1 receptor might differ. The results from the A1 knockout mouse indicate that the same receptor protein is involved at mossy fiber and nonmossy fiber synapses; however, it is possible that the local environment might change receptor properties. We have compared the affinity of the mossy fiber and Schaffer collateral A1 receptors by examining the ability of the selective A1 agonist CCPA to compete with DPCPX. We exposed slices to saturating concentrations of DPCPX (200 nM, 20 min) to relieve any endogenous adenosine tone, and then the inhibitory action of CCPA on CA1 and mossy fiber synapses was compared. As shown in Fig. 6 the two types of synapses were equally sensitive to CCPA, suggesting that the affinity of the A1 receptors is the same.

Discussion

The release characteristics of synapses vary enormously, depending on the synapse. This diversity is reflected largely in the basal probability of transmitter release, which, in turn, has dramatic effects on activity-dependent plasticity. Much of this variability

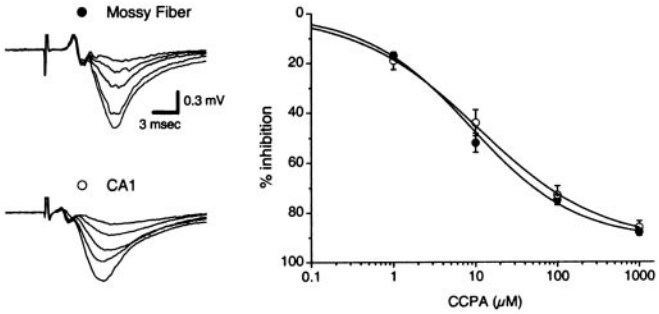

Fig. 6. Agonist affinity is similar for mossy fiber and Schaffer collateral A1 receptors. Concentration–response relationships for the A1 selective agonist CCPA were measured at mossy fiber and Schaffer collateral synapses in the presence of a saturating concentration of DPCPX (200 nM). DPCPX was applied ≥20 min before and during CCPA application to relieve any endogenous adenosine tone. The EC_{50} was 9 ± 2 μM (n = 5) for mossy fiber synapses and 11 ± 5 μM (n = 6) for Schaffer collateral synapses (P = 0.74).

is thought to arise from intrinsic differences in the release properties of different classes of synapses. In some cases, presynaptic autoreceptors can contribute to short-term plasticity at a particular synapse (19–22). A striking example of the difference in release properties of synapses is provided by a comparison of hippocampal mossy fiber synapses with the other synapses in the hippocampus. In contrast to other synapses, mossy fiber synapses show pronounced PPF and frequency facilitation and a presynaptic form of LTP (23). A prerequisite for these unique properties is low-basal-release probability at these synapses. We have found that low-release probability is not an intrinsic property of mossy fiber synapses, but rather is imposed on the synapse by tonic activation of presynaptic A1 receptors by ambient adenosine. Three separate series of experiments establish the striking and selective actions of endogenous adenosine on the mossy fiber synapses. Blockade of A1 adenosine receptors, enzymatic degradation of extracellular adenosine, and the genetic deletion of the A1 receptor caused a dramatic and selective enhancement in mossy fiber synaptic responses. Both short-term plasticity (PPF and frequency facilitation) and long-term potentiation are severely impaired in the absence of the action of ambient adenosine. Note that some LTP remains in the absence of adenosine tone. This residual component presumably accounts for the LTP that can be recorded in hippocampal granule cell autaptic cultures (24), where the levels of adenosine are presumably low.

Although our studies have been carried out in acute-slice preparations, we would argue strongly that the effects we report are independent of the technique used. The magnitude of the DPCPX effect was remarkably constant, regardless of the slicing procedure, the temperature, and the time that elapsed between slice preparation and recording. Similarly, frequency facilitation was not affected by the type of slicing procedure. Finally, *in vivo* microdialysis experiments report concentrations of extracellular adenosine in the range of 0.1 to 1 μM (25, 26), which is in the same range as calculated in the slice (27). It will be of interest to see how the distinctive properties of the mossy fiber synapse participate in information processing in the intact animal.

Why are the effects of ambient adenosine so prominent on mossy fiber synapses when compared with other synapses in the hippocampus? We have considered five possibilities. (*i*) Endogenous adenosine acts on different receptor subtypes, (*ii*) the density of A1 receptors is higher on mossy fiber synapses, (*iii*) the affinity of A1 receptors on mossy fiber synapses is higher than on other synapses, (*iv*) mossy fiber A1 receptors are coupled more effectively to their downstream effectors, and (*v*) the concentration of adenosine is higher around mossy fiber synapses. First, it is unlikely that different adenosine receptors are involved. All the effects of ambient adenosine can be attributed to the activation of presynaptic A1 receptors on mossy fiber terminals, because the enhancement in mossy fiber transmission caused by removal of extracellular adenosine by adenosine deaminase or by blocking A1 receptors by DPCPX (data not shown) is not observed in A1 receptor knockout mice. Second, it seems unlikely that the differences in the effect of endogenous adenosine are due to differences in the A1 receptor density. Although autoradiographic studies on the distribution of A1 receptors (28, 29) do not specifically address the density of A1-binding sites in stratum lucidum, no obvious increased density is apparent in the material illustrated. Third, we find that synapses with high (CA1 synapses) and low (mossy fiber synapses) basal release probability are equally sensitive to inhibition by an A1-selective agonist when the effects of endogenous adenosine are blocked (see Fig. 6). Therefore, a difference in affinity of the receptor is unlikely to explain the difference. Thus, based largely on a process of elimination, differences in receptor coupling or local high levels of adenosine in stratum lucidum most likely account for our results. Extracellular adenosine is derived from several sources, the most prominent being the metabolism of ATP (9). This conversion is accomplished by extracellular 5′-nucleotidase. Immunocytochemical studies have revealed a high level of this enzyme in stratum lucidum (30). Alternatively, adenosine could be released by equilibrative transport if intracellular concentrations are high. Dipyridamole, an inhibitor of equilibrative transporters, depresses rather than increases mossy fiber fEPSPs (data not shown), suggesting that transport does not contribute adenosine release in stratum lucidum under basal conditions.

In conclusion, we have found that the defining physiological features of mossy fiber synapses are imposed in large part by the tonic action of extracellular adenosine acting on presynaptic A1 receptors. These results emphasize that the local extracellular environment can play a critical role in sculpting the dynamic properties of synapses.

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